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Title:
Method and composition for retarding staling of bakery products by adding a thermostable protease

Abstract:

The present invention is related to a method for the prevention or retarding of staling during the baking process of bakery products which comprises the step of adding a sufficiently effective amount of at least one intermediate thermostable or thermostable protease in said bakery products. The present invention further relates to an improver for the prevention or retarding of staling during the baking process of bakery products, characterised in that it comprises at least one intermediate thermostable or thermostable protease.

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(54) Method and composition for retarding staling of bakery products by adding a thermostable protease

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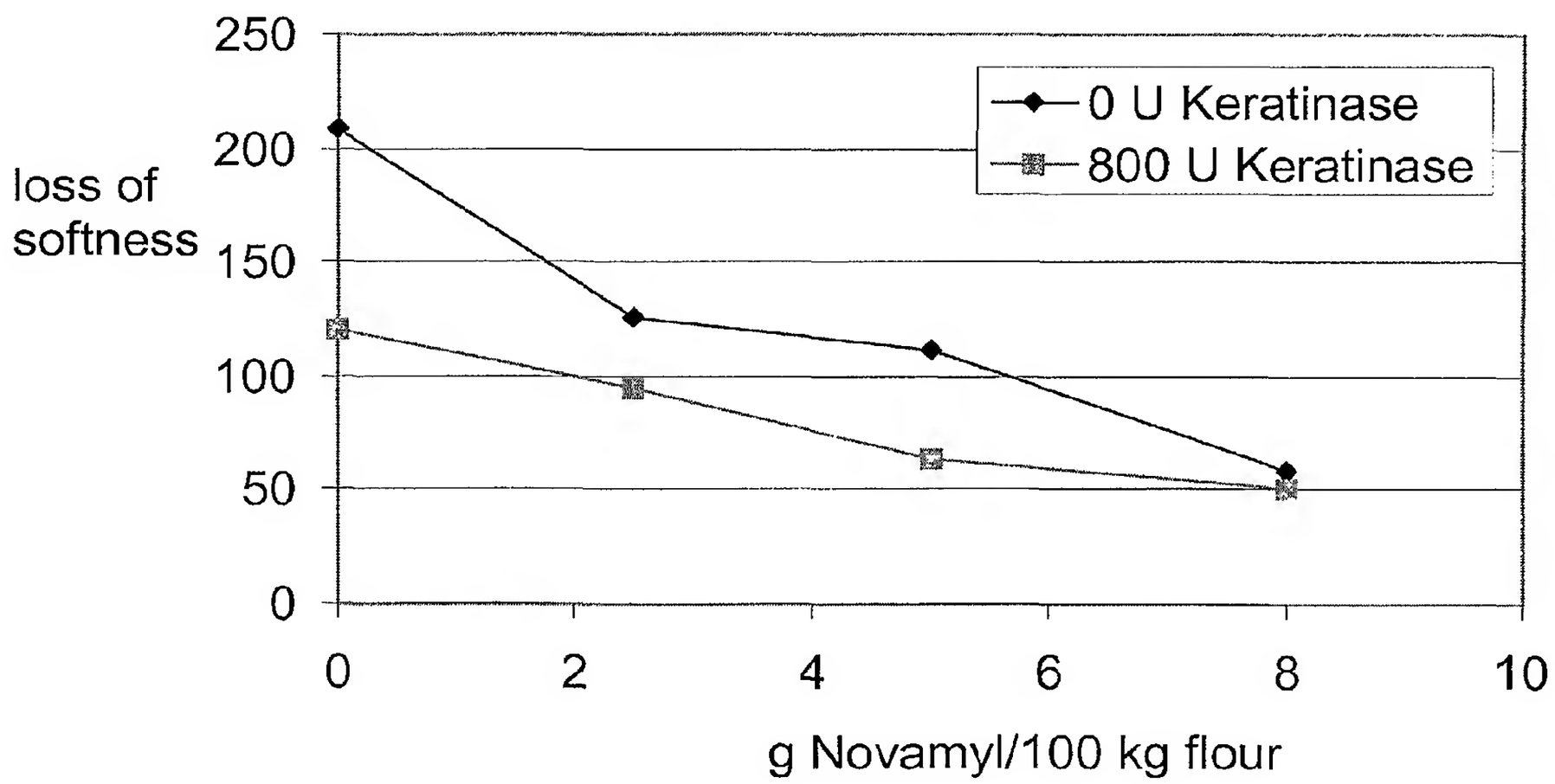

Fig. 3

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Description

Field of the invention

[0001] The present invention concerns a method and a composition for the prevention or retarding of staling and associated effects during the baking process of bakery products which comprise at least one thermostable protease.

Background of the invention

[0002] The consumers prefer to buy fresh bread and they want it to remain fresh for a long time. Retarding the staling has always been a challenge to producers of bakery ingredients. The fact that the production of bread is more and more centralised and farther away from the distribution points puts an even larger pressure on the development of additives and ingredients to maintain the softness of bread. Also soft rolls, hamburger, buns and pastry products are subject to staling and a loss of softness. There are a number of ingredients known to retard the staling of bread and soft bakery products. Fat and emulsifiers such as distilled monoglycerides and stearoyllactylates are already used since decades. Mono-, di- and polysaccharides have a positive influence on water retention and binding. Water loss is often associated with staling and the saccharides have positive influence on the mouthfeel of baked products and thus diminish the perception of staling. Amylases are known to have a beneficial effect on staling and starch retrogradation.

[0003] Bread staling is a complex phenomenon. It is perceived as a softening of the crust, a hardening of the crumb and the disappearance of fresh bread flavour. The hardening of the crumb is not only due to a loss of water during storage as was already demonstrated by Boussingault in ((1852) Ann. Chim. Phys. 3,36,490). It is the result of a number of physico-chemical processes. Over the years, researchers have tried to unravel these processes and developed different theories.

[0004] In the early days, bread firming was attributed solely to the retrogradation of starch (Katz, J.R. (1930) Z. Phys. Chem., 150, 37-59). It was shown by X-ray diffraction that the starch in bread is forming a micro-crystalline structure during storage. Later it was shown that the water soluble starch fraction diminished during bread staling (Schoch et al. (1947) Cereal Chem., 24, 231-249), which concludes that during baking starch granules absorb water. The linear amylose chains become soluble and diffuse to the water phase. In time more and more amylose is present in the water phase. So the amylose is partially leached out of the swollen starch granules. The branched amylopectine remains in the granules. The leaching process is limited by the available water. During cooling the amylose retrogrades very quickly and forms a gel. The retrogradation of amylopectine is believed to involve primarily association of its outer branches and requires a longer time than does the retrogradation of amylose, giving it prominence in the staling process, which occurs over time after the product has cooled, aggregate more slowly, due to stereochemical interferences. The amylopectine formed intramolecular bonds. The prominent role of starch in staling of bread is further illustrated by the use of carbohydrases to diminish or to slow down the staling of baked products. It was shown (Conn J. F. et al. (1950) Cereal Chem., 27, 191-205) that amylases from bacterial or fungal origin slow down the rate of staling of bread and result in a less firm crumb structure. The addition of thermostable alfa-amylases or beta-amylases is most effective. However this also results in a gummy and sticky crumb.

[0005] The document EP0412607 discloses the use of a thermostable alfa-1,6-endoglucanase or an alfa-1,4-exoglucanase to reduce staling; EP0234858 discloses the use of a thermostable maltogenic beta-amylase to retain the crumb softness.

[0006] However, it is still not clear whether the anti-staling effect is due to the dextrins produced or to the modification of the amylose and amylopectine and the consequent reduced tendency to crystallise. Also the influence of emulsifiers as glycerolmonostearate and sodiumstearoyllactylate seems to confirm the role of starch in bread crumb firming (Schuster G. (1985) Emulgatoren für Lebensmittel - Springer Verlag 323-329). It is the interaction between these emulsifiers and the starch which results in a changed starch conformation that accounts for the observed reduction of staling.

[0007] As there was not always a good correlation between starch structure and staling (Zobel H.F. et al (1959) Cereal Chem., 36, 441), other flour constituents were also investigated. The role of flour proteins in the crumb firming process has been studied but it was found that they were less important than starch (Cluskey, J.E. (1959) Cereal Chem., 36, 236-246.), (Dragsdorf, R.D. et al. (1980) Cereal Chem., 57, 310-314) studied the water migration between starch and gluten during bread storage. These authors concluded that due to a change in the cristallinity of the starch, it adsorbed more water, so the water migrates from the gluten to the starch and so less free water is available.

[0008] In later study (Martin et al. (1991) Cereal Chem., 68(5), 498-503 and 503-507), it appears that the high molecular weight dextrins do not have an antifirming effect on bread crumb. Instead, the high DP dextrins may entangle and / or form a hydrogen bond with protein fibrils, thus effectively cross-linking the gluten. Consequently, the firming rate is increased. It is stated that in weaker flours the gluten interacts stronger with the starch granules. This result in bread crumb that firms faster. However better gluten quality and stronger flour also result in higher loaf volume and

thus in a softer crumb. Axford et al. (1968) cited in Faridi, H. (1985) Rheology of wheat products, AACC, p. 263-264) showed that the loaf specific volume was a major factor in measuring both the rate and the extent of firming. So the role of gluten in bread firming remains still questionable and few attempts have been made to slow down firming based on gluten modification.

[0009] Proteases have a long history of use in the baking sector. They are mostly used by the baker to reduce mechanical dough development requirements of unusually strong or tough gluten. They lower the viscosity and increase the extensibility of the dough. In the end product they improve the texture compressibility, the loaf volume and the bread colour. Also the flavour can be enhanced by production of certain peptides. The proteases mellow the gluten enzymatically rather than mechanically. They reduce the consistency of the dough, decreasing the farinograph value. The proteases most used in baking are from Aspergillus oryzae and Bacillus subtilis. The neutral bacterial proteases are by far more active on gluten than the alkaline proteases. Papain, bromelain and ficin are thiol-proteases extracted from papaya, pineapple and figs. Especially papain is very reactive towards gluten proteins. Bacterial proteases and papain, especially neutral proteases, are used in cookies, breadsticks and crackers where a pronounced slackening of the dough is wanted. However, in breadmaking, a more mild hydrolysis of fungal proteases is preferred.

[0010] Proteases also have major disadvantages. The action of the proteases is not limited in time, it continues after mixing and weakens the dough structure in time. This phenomenon increases the risk of weakening the dough and increases the stickiness of the dough. Sometimes their action is even enhanced by the pH drop during fermentation. The use of proteases in baking requires strict control of the bulk fermentation and proofing conditions of the dough. The proteases are inactivated during baking (Kruger, J.E. (1987) Enzymes and their role in cereal technology AACC 290-304). Especially neutral Bacillus proteases and papain should be dosed very carefully as overdoses slacken the dough too much. This may result in dough collapse before ovening or a lower bread volume and a more open crumb structure. Especially in Europe, where the flours are weaker than in the US or Canada, the risk of overdosing protease is very present.

[0011] Furthermore, proteases also increase stickiness because by the hydrolytic action water is released from the gluten (Schwimmer, S. (1981) Source book of food enzymology-AVI Publishing, 583-584). This means that in practice proteases are not much used in breadmaking in Europe.

[0012] The document EP021179 discloses the use of an alfa-amylase preparation in which the protease (inactivated) was used in combination with emulsifiers to inhibit staling.

[0013] Conforti et al. (1996) FSTA, 96(12), M0190 Abstract of presentation) added an enzyme mixture comprising bacterial amylase, fungal amylase and fungal protease to fat substituted muffins. The control fat containing muffin was more tender. The enzyme treatment decreased the staling rate. This is not surprising in view of the presence of amylases.

[0014] Lipase is also known to soften bread crumb and to somewhat reduce the firming rate of bread crumb (WO 94/04035 example 2).

[0015] Fungal proteases are sensitive to high temperatures. Some bacterial neutral and alkaline protease are resistant to higher heat treatments. Neutral thermostable proteases from Bacillus, which may be tolerant to oxidising agents, are preferred in detergent formulations. Also alkaline thermostable proteases from Bacillus are used in washing and detergent formulations. Papain is very heat stable and requires a prolonged heating at 90 - 100°C for deactivation. Bromelain is less stable and can be deactivated at around 70°C. Other heat stable proteases are produced by Bacillus licheniformis NS70 (Chemical Abstracts, 127, 4144 CA), Bacillus licheniformis MIR 29 (Chemical Abstracts, 116, 146805 CA), Bacillus stearothermophilus (Chemical Abstracts, 124, 224587 CA), Nocardiopsis (Chemical Abstracts, 114, 162444 CA) and Thermobacteroides (Chemical Abstracts, 116, 146805 CA). This is not an exhaustive list, but it illustrates the importance of the thermostable proteases and their application, mostly in detergents. No reference is made to baking and anti-staling properties.

State of the art

[0016] Keratinase is a protease which is active on keratin, a scleroprotein existing as a constituent in mammalian epidermis, hair, wool, nails and feathers. Practical applications of the enzyme are as ingredient in depilatory compositions; as dehairing aid of hides in leather manufacture, the breaking down of keratin and reconstitution into textile fabrics. No application of said enzyme in the food industry is known.

[0017] Thermus aquaticus is a hyperthermophile belonging to the Archea. The well known "Taq polymerase" is isolated from this organism. Pyrococcus furiosus is another representative from this group. Thermostable proteases were isolated from these organisms.

Summary of the invention

[0018] A first aspect of the present invention is related to a method for the prevention or retarding of staling and

associated effects during the baking process of bakery products, said method comprising the step of adding a sufficiently effective amount of at least one thermostable protease to the ingredients of said bakery products.

[0019] Preferably, the thermostable protease has its optimal temperature activity higher than 60°C, preferably higher than 70°C, more preferably higher than 80°C. The preferred thermostable protease used in the method according to the invention presents a ratio between the protease activity at optimum temperature and the protease activity at 25°C, higher than 10, preferably higher than 15.

[0020] Such thermostable protease can be obtained by extraction from naturally occurring eukaryotic or prokaryotic organisms, by synthesis or by genetic engineering by a method well-known to a person skilled in the art.

[0021] The preferred thermostable protease is Taq protease which can be advantageously isolated from the strain Thermus aquaticus (LMG8924) or is keratinase, preferably isolated from Bacillus licheniformis (LMG7561).

[0022] In the method according to the invention, use of the thermostable protease can be combined with another enzyme, such as a thermostable α-amylase, β-amylase, intermediate thermostable maltogenic amylase, lipase, glycolsyltransferases or pullulanases. The thermostable protease can also be added to a non-enzymetic additive such as an emulsifier (monoglyceride, diglyceride and/or stearoyllactylades). Other suitable emulsifiers may also be added to said thermostable protease during the baking process. Synergistic or cumulative effects are present.

[0023] Therefore, the method according to the invention will result in improved bakery products which are preferably selected from the group consisting of bread, soft rolls, bagels, donuts, danish pastry, hamburger rolls, pizza, pita bread and cakes.

[0024] In the method according to the present invention, the sufficiently effective amount of protease lies preferably between 150 and 5000 U /100 kg flour, more preferably between 250 and 2000 U /100 kg flour, and advantageously between 400 and 1000 U /100 kg flour.

[0025] Another aspect of the present invention is related to an anti-staling composition for bakery products comprising at least one thermostable protease.

[0026] Another embodiment of the present invention is an improver composition comprising at least one intermediate thermostable or thermostable protease. An improver composition is a well-known concept amongst bakers. It is a mixture of active ingredients such as enzymes and emulsifiers, which are mixed with the usual ingredients for making bread, such as flour and water.

Detailed description of the invention

[0027] The invention relates to the use of an intermediate thermostable or thermostable protease in baked goods. The enzyme preparation has a pronounced effect on crumb softness and on retarding the staling of baked products. The enzyme preparation is characterised by the fact that it has no adverse effect on dough rheology, on the crumb structure and on the volume of the resulting bread. The enzyme has a low activity at a temperature of 25°C to 40°C. The enzyme has a temperature optimum of 60 - 80°C or higher. The enzyme is or is not inactivated during the baking process. The thermostable proteases are characterised by having a positive effect as anti-staling agents. This effect is especially noticeable in combination with other anti-staling enzymes. As examples of other anti-staling enzymes the person skilled in the art may select thermostable amylases from Bacillus licheniformis or Bacillus stearothermophilus and thermostable maltogenic amylases (i.e. Novamyl ®from Novozymes). Their effect is also additive to the anti-staling effect of mono- en diglycerides, stearoyllactylates and other emulsifiers used in baking.

[0028] The thermostable proteases can be used in bread, soft rolls, bagels, donuts, danish pastry, hamburger rolls, pizza and pita bread, cake and other baked products where staling and inhibition thereof is an quality issue. The thermostable protease can be produced by prokaryotes (bacteria) and eukaryotes (fungi, Archea).

[0029] Basically the most important characteristics of the proteases that are used in this invention are:

1) Their thermostability: At a pH where the enzyme is stable they have a temperature optimum that is higher than 60°C, preferably higher than 70°C and even more preferable higher than 80°C
2) The ratio between the activity at optimum temperature and at 25 °C is at least higher than 10 and preferable higher than 15.

[0030] The enzyme is preferably a keratinase or Taq protease. The keratinase is preferable produced by *Bacillus* licheniformis (example *B.* licheniformis LMG 7561). The Taq protease is preferable produced by *Thermus aquaticus* (example *Thermus aquaticus* LMG 8924).

[0031] The proteases may be obtained from the respective micro-organisms by use of any suitable technique. For instance, the protease preparation may be obtained by fermentation of a micro-organism and subsequent isolation of the protease containing preparation from the resulting broth by methods known in the art such as centrifugation and ultrafiltration. The proteases may also be obtained by cloning the DNA sequence coding for a suitable protease in a host organism, expressing the protease intra- or extra-cellular and collecting the produced enzyme.

[0032] The proteases may also be obtained by directed evolution or gene shuffling of thermostable or non-thermostable proteases or enzymes. As long as they have peptide cleaving activity, they are considered to be proteases in the scope of this invention.

[0033] Surprisingly, the inventors found that the use of a protease which had no perceivable action on the dough rheology had a pronounced effect on the softness and retardation of the crumb hardness. There was no adverse effect on the crumb elasticity or no increase of the crumb stickiness as compared to a control. The effect was additive to known anti-staling agents (such as α-amylases) and permits the development of bread and other soft bakery products with a prolonged shelf life.

[0034] The choice of the protease is very important. The protease should exert no adverse effect during mixing and the subsequent proofing. Otherwise the dosage that can be administered is to low to diminish the staling rate and to maintain a good crumb elasticity. The higher the temperature optimum of the enzyme the lower the negative effect on the crumb structure and on the dough rheology.

[0035] The present invention will be described hereafter in detail in the following non-limiting examples and in reference to the enclosed figures.

Short description of the figures

[0036] The figure 1 represents the optimum activity of protease at pH 7.0, in a buffered solution of 0.1 M phosphate for aqualysin and keratinase.

[0037] The figure 2 represents the retarding effect of the addition of Taq protease upon staling of bread.

[0038] The figure 3 shows the improved effect on retarding bread staling following the addition of keratinase in bread.

Description of a preferred embodiment of the invention

[0039] The preferred protease used, is obtained from the strain *Bacillus licheniformis* LMG 7561 and has keratinase activity. The keratinase in question is obtained by culturing the strain Bacillus licheniformis LMG 7561 on the following medium: 0.5 g/l NH_4Cl, 0.5 g/l NaCl, 0.3 g/l K_2HPO_4, 0.4 g/l KH_2PO_4, 0.1 g/l $MgCl_2.6H_2O$, 2 g/l citric acid, 0.1 g/l yeast extract and 10 g/l feather meal. The medium is adjusted to pH 6.5 with phosphoric acid. No pH control is imposed. Incubation is done at 45°C with aeration (p_2 60%, 1.25 vvm) during 40 hours after which the medium is collected for further concentration. The supernatant is then concentrated by membrane ultrafiltration (molecular cut off 5000 Da). The crude keratinase solution obtained that way is stored frozen until used in baking tests.

[0040] The keratinase solution obtained that way displays maximum activity at a temperature of 60 °C and a pH of 8.0. In the pH range of 7 to 9 more than 85% of the maximum activity was measured. There isn't almost any loss of enzyme activity while heating the solution an hour at 60°C. Heating the enzyme at 70°C during 14 min reduces the activity with 50%.

[0041] The activity was measured on keratin. For standard measurements, 4 g of keratin were dissolved in 100 ml sodium hydroxide. After dissolution the pH is adjusted slowly to 8.0 with 3.2 M phosphoric acid. Distilled water is added to a final volume of 200 ml. 5 ml of the substrate solution is pre-incubated at 60°C. 1 ml of enzyme solution is added and incubated at 60°C. Then 5 ml of 14% TCA (TriChloroAcetic acid) is added to the incubated enzyme solution. Mixed for 60 minutes. The solution is filtered and the absorbance is measured at 275 nm relative to a blank solution (enzyme added after the TCA addition).

The activity is expressed as KU/ml

$$= \frac{(A275nm\ Enzyme - A275nmBlanc)*11}{0.0075 * 30}$$

The fermentation contained 300 to 1500 KU/ml.

[0042] For baking purposes the activity was expressed as mU/ml based on the protazym tablet determination. The KU were only used to demonstrate the presence of the keratinase.

[0043] The Taq protease in question is obtained by culturing the strain *Thermus aquaticus* LMG 8924 on the following medium : 1 g/l tryptone; 1 g/l yeast extract; 100 ml/l salt solution and 900 ml distilled water. The pH is adjusted to 8.2 with 1 M NaOH prior to sterilisation 121°C for 15 minutes. The salt solution has the following composition : 1 g/l nitriloacetic acid 0.6 g/l $CaSO_4.2H_2O$; 1 g/l $MgSO_4.7H_2O$; 80 mg/l NaCl, 1.03 g/l KNO_3; 6.89 g/l $NaNO_3$; 2.8 g/l $Na_2HPO_4.12H_2O$; 10 ml/l $FeCl_3.6H_2O$ solution (47 mg/100 ml); 10 ml/l Trace element solution and 1 distilled water. The Trace element solution has the following composition : 0.5 ml/l H_2SO_4; 1.7 g/l $MnSO_4.H_2O$; 0.5 g/l $ZnSO_4.7H_2O$; 0.5 g/l H_3BO_3; 25 mg/l $CuSO_4.5H_2O$; 25 mg/l $Na_2MoO_4.2H_2O$; 46 mg/l $CoCl_2.6H_2O$ and 1 l distilled water. Incubation is done at 60°C with aeration (pO_2 60%, 4 vvm) during 24 hours after which the medium is collected for further concentration. *Thermus aquaticus* LMG 8924 produced at least two kinds of extracellular proteases. One of the extracellular proteases

was called aqualysin I, and is an alkaline protease which was secreted linearly from the early stationary phase until the time the cells ceased to grow. The optimum temperature of the proteolytic activity was between 70 and 80°C. The other was called aqualysin II and is a neutral protease, the production of which appeared from day 4 and the concentration of this protease continued linearly for 5 days. The maximum activity was obtained at 95°C (the highest temperature tested). We used the fermentation extract after 1 day of fermentation for the baking tests. As we stopped the fermentation after 1 day the protease present is the aqualysin I.

[0044] The supernatant is then concentrated by membrane ultrafiltration (molecular cut off 10000 Da). The crude Taq protease solution obtained that way is stored frozen until used in baking tests.

[0045] The Taq protease solution obtained that way displays maximum activity at a temperature of 80°C. There isn't almost any loss of enzyme activity while heating the solution an hour at 80°C. Heating the enzyme at 90°C during 10 min reduces the activity with 60%.

[0046] The protease activity was measured on azurine-crosslinked casein (AZCL-casein). It is prepared by dyeing and crosslinking casein to produce a material which hydrates in water but is water insoluble. Hydrolysis by proteases produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity (Protazyme AK Tablets, Megazyme, Ireland). A protazyme AK tablet is incubated in 100 mM $Na_2HPO_4.2H_2O$; pH 7.0 at 60°C for 5 min. An aliquot of enzyme (1.0 ml) is added and the reaction is allowed to continue for exactly 10 min. The reaction is terminated by the addition of tri-sodium phosphate (10 ml, 2% w/v, pH 12.3). The tube is allowed to stand for approx. 2 min at room temperature and the contents are filtered. The absorbance of the filtrate is measured at 590 nm against a substrate blank.

The activity is expressed as

$$mU/ml = (34.2 * (Abs_{590}\ enzyme - Abs_{590}\ blank) + 0.6) / dilution$$

[0047] Side-activities like α-amylase activity was measured by the Phadebas Amylase Test (Pharmacia & Upjohn). The substrate is a water-insoluble cross-linked starch polymer carrying a blue dye. It is hydrolysed by α-amylase to form water-soluble blue fragments. The absorbance of the blue solution is a function of the α-amylase activity in the sample.

[0048] Xylanase side-activity was measured by the Xylazyme Method (Megazyme). The substrate employed is azurine-crosslinked xylan. This substrate is prepared by dyeing and crosslinking highly purified xylan (from birchwood) to produce a material which hydrates in water but is water insoluble. Hydrolysis by endo-(1,4)-β-D-xylanase produces water soluble dyed fragments, and the rate of release of these (increase in absorbance at 590 nm) can be related directly to enzyme activity.

[0049] The Taq protease solution obtained didn't show any α-amylase or xylanase side activity.

[0050] The keratinase solution obtained had no xylanase activity and contained less than 8 U/ml α-amylase activity as measured by Phadebas test.

[0051] The baking tests were performed in 1 kg bread. The basic recipe was:

Flour (Duo) :	1500
Water :	840
Fresh Yeast (Bruggeman, Belgium) :	75
Sodium Chloride :	30
Partially hydrogenated palm oil	21
Distilled monoglycerides	3
saccharose	6
Ascorbic acid	0.06

[0052] The following breadmaking process was used : The ingredients were mixed for 2' at low and 6' at high speed in a Diosna SP24 mixer. The final dough temperature was 29°C. After bulk fermentation for 20' at 25°C, 600 g dough pieces were made up using the Euro 200S (Bertrand-Electrolux Baking) set at R8/L19 and moulded. The dough pieces are proofed at 35°C for 50' at 95% relative humidity. Then the breads are baked at 230°C in a MIWE CONDO (Micheal Wenz - Arnstein - Germany) oven with steam (0.1 L before and 0.2 L after ovening the breads). It is obvious to one skilled in the art that same end results can be obtained by using equipment of other suppliers.

[0053] The softness of the bread was measured by a TA-XT2 texture analyser (Stable Micro Systems UK). The bread was sliced and the force to obtain a 25% deformation of 4 slices of 1cm was measured. This is called the hardness.

The hardness is measured at day 1 and day 6 after baking. The difference between the two measure forces is "the loss of softness":

$$\text{Loss of softness} = \frac{\text{deformation force at day 6 - deformation force at day 1}}{\text{force at day 1}}$$

[0054] It is a relative measure. The absolute values have no meaning as such but should be compared to a reference for interpretation.

[0055] The elasticity is the difference between the aforementioned force and the force after 20 sec of relaxation. When the elasticity is lower than in the reference bread, this means that the crumb becomes less resilient. The crumb, when compressed does not regain its original shape. This means that during slicing or handling the crumb structure may be lost irreversibly. It is important that by using an added enzyme there is no loss of elasticity compared to a control bread

Examples

Example 1: Effect or keratinase and Taq protease on Proof Time, Loaf Moisture and Specific Loaf Volume

[0056] Additions of either enzyme to the bread dough did not change the proof time, loaf moisture and specific load volume. The initial moisture content of bread varied slightly, but all the loaves lost approximately the same amount of moisture during six days of storage at room temperature.

Example 2

[0057] A bread has been baked according to the aforementioned method with addition of Taq protease (eventually in the presence of different doses of Novamyl 10 000 BG from Novozymes (Denmark)).

[0058] Doses are expressed on 100kg of flour weight used in the baking test.

[0059] The following table 1 expresses the loss in softness between day 1 and day 6 after baking as defined previously.

Table 1

Novamyl g/100kg	800 U Taq protease	0 U Taq protease
0	140	209
2.5	128	152
5	96	118
8	68	105

[0060] The example shows that the use of Taq protease will retard staling in bread. A combination of Taq protease with an intermediate thermostable maltogenic amylase (e.g. Novamyl®, commercial enzyme of Novozymes) will retard staling in bread significantly. So there is a synergistic effect between the thermostable proteases and α-amylases. This effect becomes more pronounced at higher doses of Novamyl (see fig.2).

[0061] Table 2 shows that the elasticity of the bread crumb is hardly affected by the use of the Taq protease.

Table 2:

Elasticity		
Novamyl g/100kg	800 U Taq protease	0 U Taq protease
0	61.5	61.9
2.5	63.1	63.4
5	63.0	64.2

Table 2: (continued)

Elasticity		
Novamyl g/100kg	800 U Taq protease	0 U Taq protease
8	63.4	64.9

Example 3

[0062] A bread baked according to the aforementioned method with the addition of keratinase (eventually in the presence of Novamyl 10 000 BG from Novozymes (Denmark)).

[0063] Doses in the following table 3 are expressed on 100kg of flour weight used in the baking test.

[0064] The table expresses the loss in softness between day 1 and day 6 after baking as defined previously.

Table 3

Novamyl g/100 kg	800 U keratinase	0 U keratinase
0	121	209
2.5	95	126
5	64	111
8	50	59

[0065] It is clear from this experiment that adding the thermostable protease keratinase has a pronounced effect on softness. There is a cumulative effect with thermostable maltogenic amylases as Novamyl. It was verified that the small quantity of amylase present in the preparation had no impact on softness and the relaxation ratio by testing this amylase separately.

[0066] Table 4 also shows that there is no adverse effect on the relaxation ratio when this protease is used.

Table 4

Novamyl g/100kg	800 U keratinase	0 U keratinase
0	62.6	63.6
2.5	64.3	65.0
5	65.5	65.8
8	65.4	65.8

[0067] Treatment with keratinase or Taq protease alone or together with thermostable amylases (e.g. Novamyl®) significantly affects bread softness. The enzyme treated bread was softer, when Taq protease or keratinase were added. The examples illustrate the invention that thermostable proteases increase shelf live of baked products as far as softness and staling are concerned.

Example 4: Effect of keratinase and Taq protease on the crumb structure and the sensory characteristics of bread

[0068] These thermostable proteases didn't have a negative effect on the crumb structure like other non thermostable proteases, where the crumb structure becomes more open, dependent of the doses that have been used. There was also no effect on the volume of the baked products by using these thermostable proteases.

[0069] Crust colour, character of crust, colour of crumb, aroma and taste of bread did not change significantly with the addition of keratinase or Taq protease.

Claims

1. Method for the prevention or retarding of staling during the baking process of bakery products which comprises the step of adding a sufficiently effective amount of at least one intermediate thermostable or thermostable protease in said bakery products.

2. Method according to claim 1, **characterised in that** the thermostable protease has a temperature activity optimum higher than 60°C.

3. Method according to the claim 1 or 2, **characterised in that** the ratio between the protease activity at optimum temperature and the protease activity at 25°C is higher than 10, preferably higher than 15.

4. Method according to any one of the preceding claims, **characterised in that** the thermostable protease is obtained by extraction from naturally-occurring eukaryotic or prokaryotic organisms, by synthesis or by genetic engineering.

5. Method according to any of the preceding claims, **characterised in that** the thermostable protease is a Taq protease, preferably isolated from Thermus aquaticus LMG 8924, and/or a keratinase, preferably isolated from Bacillus licheniformis LMG 7561.

6. Method according to claim 5, **characterised in that** the thermostable protease is selected from the group consisting of aqualysin I, aqualysin II and keratinase.

7. Method according to any of the preceding claims, further comprising the step of adding another anti-staling additive selected from the group consisting of thermostable α-amylase, β-amylase, intermediate thermostable maltogenic amylase, lipase, glycolsyltransferases, pullulanases and emulsifiers, preferably monoglycerides, diglycerides and/or stearoyllactylates.

8. Method according to any one of the preceding claims, **characterised in that** the sufficiently effective amount of protease lies preferably between 150 and 5000 U /100 kg flour, more preferably between 250 and 2000 U /100 kg flour, and advantageously between 400 and 1000 U /100 kg flour.

9. Method according to any one of the preceding claims, **characterised in that** the bakery product is selected from the group consisting of bread, soft rolls, bagels, donuts, Danish pastry, hamburger rolls, pizza, pita bread and cakes.

10. Improver for the prevention or retarding of staling during the baking process of bakery products, **characterised in that** it comprises at least one intermediate thermostable or thermostable protease.

11. Improver as in claim 10, **characterised in that** the protease has a temperature activity optimum higher than 60°C.

12. Improver as in claim 10 or 11, **characterised in that** the ratio between the protease activity at optimum temperature and the protease activity at 25°C is higher than 10, preferably higher than 15.

13. Improver as in any of the claims 10 to 12, **characterised in that** said protease is obtained by extraction from naturally occurring eukaryotic or prokaryotic organisms, by synthesis or by genetic engineering

14. Improver as in any of the claims 10 to 13, **characterised in that** said protease is a Taq protease and/or a keratinase.

15. Improver as in any of the claims 10 to 14, **characterised in that** said protease is selected from the group consisting of aqualysin I, aqualysin II and keratinase.

16. Improver as in any of the claims 10 to 15, **characterised in that** it further comprises another anti-staling additive selected from the group consisting of thermostable α-amylase, β-amylase, intermediate thermostable maltogenic amylase, lipase, glycolsyltransferases, pullulanases and emulsifiers, preferably monoglycerides, diglycerides and/or stearoyllactylates.

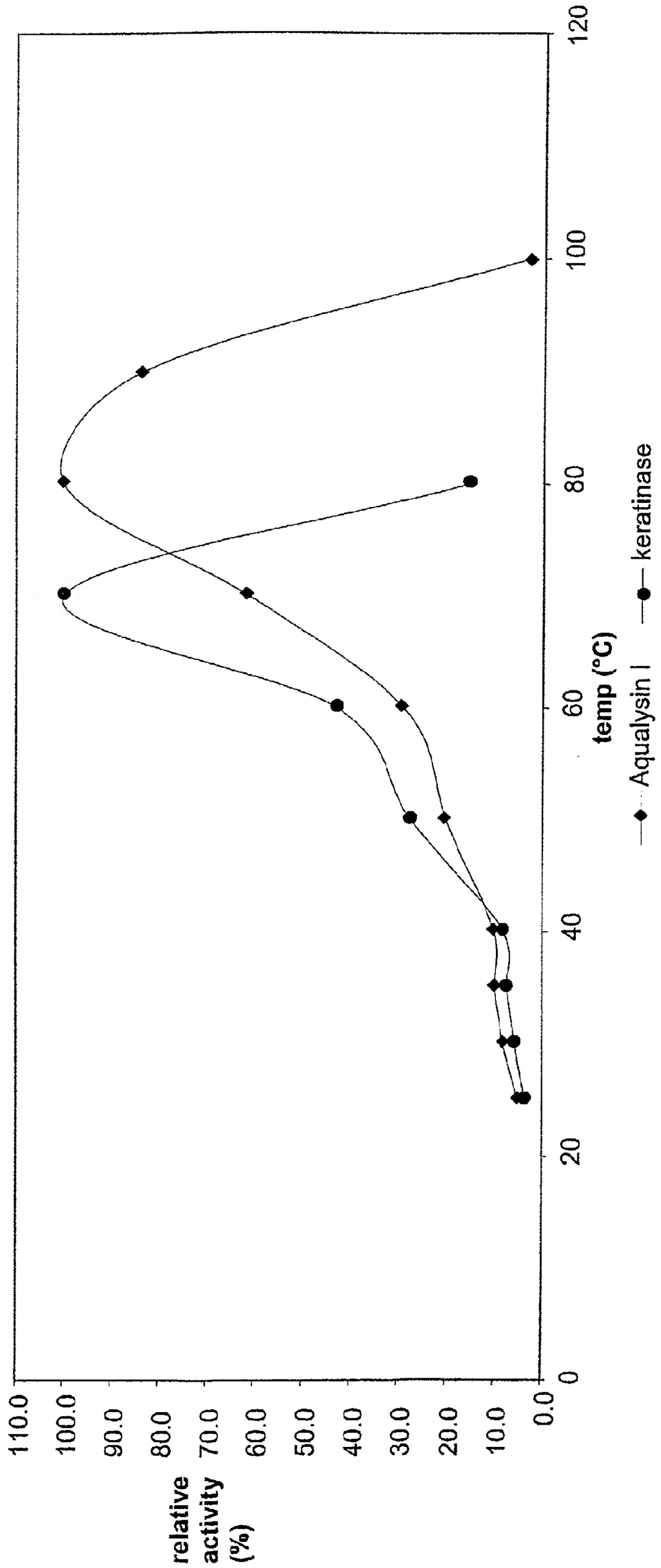

Fig. 1

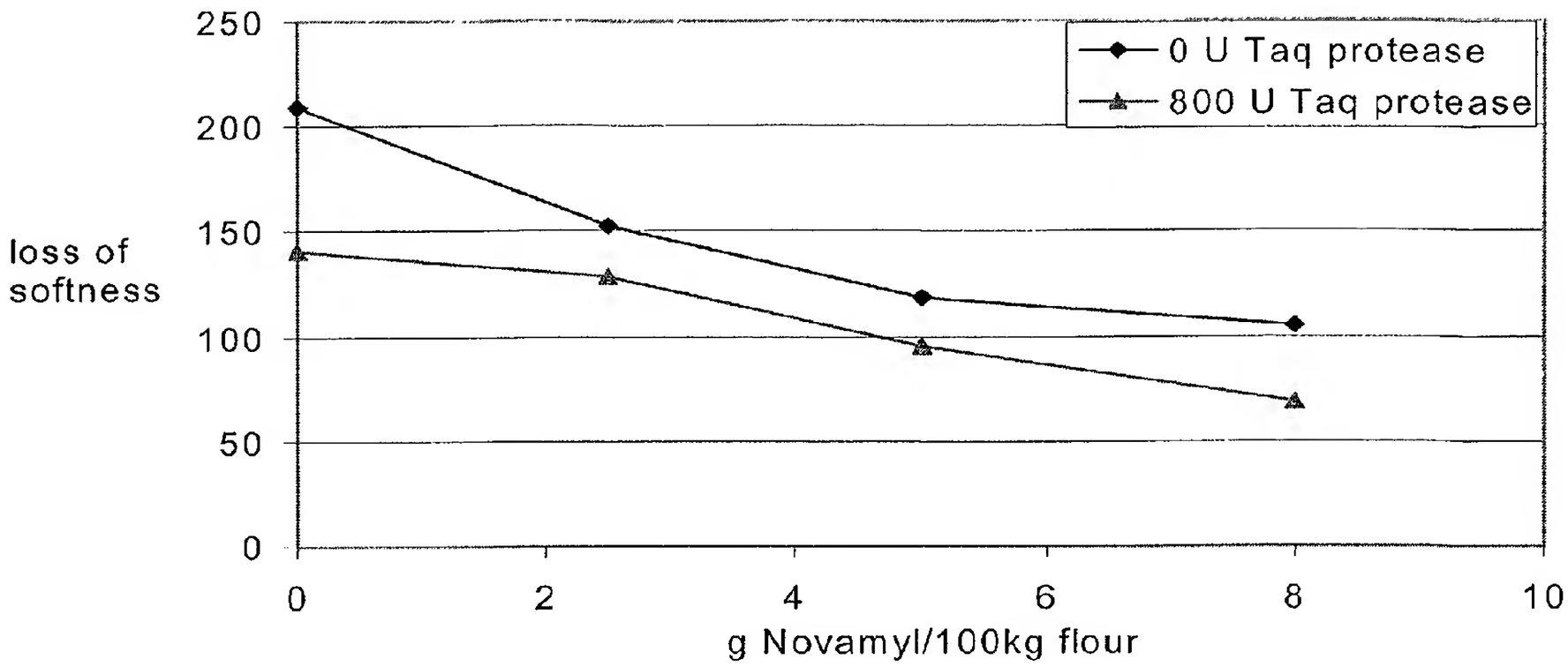

Fig. 2

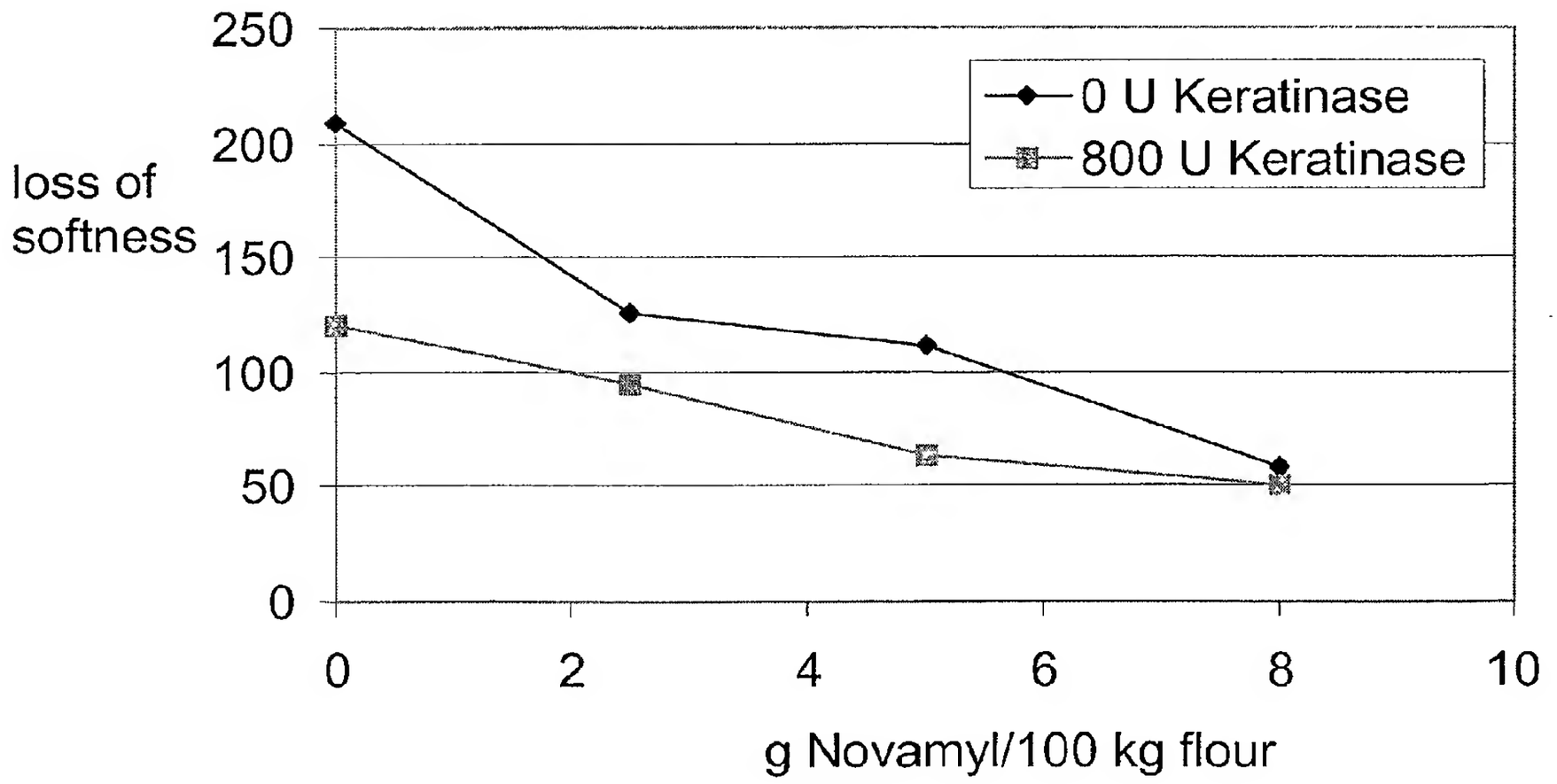

Fig. 3

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EUROPEAN SEARCH REPORT

Application Number
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The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	26 September 2002	Piret-Viprey, E

CATEGORY OF CITED DOCUMENTS

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